# Characteristics of sourdoughs and baked pizzas as affected by starter culture inoculums

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**ABSTRACT**

Previous investigations on pizza dough lactic acid bacteria (LAB) revealed that facultative heterofermentative species (FHS) were more represented than obligate heterofermentative species (OHS) within the *Lactobacillus* genus. Thus, the main hypothesis of this work was that facultative and obligate heterofermentative *Lactobacillus* species can impact differently the appreciation of baked pizza. The performances of different *Lactobacillus*, including *L. sanfranciscensis*, *L. brevis* and *L. rossiae* among FHS and *L. plantarum*, *L. graminis* and *L. curvatus* among OHS were tested in single or multiple combinations during pizza production. The values of pH, total titratable acidity and LAB levels indicated that the acidification process was almost comparable among trials. The fermentation quotient of FHS trials was above 4.0. All trials were dominated by the added LAB and for the trials with the multiple strain starter inocula, the species found at the highest cell densities were *L. sanfranciscensis*, *L. brevis* and *L. plantarum*. Significant differences among pizzas were found for weight loss, colour, morphology and volatile organic compounds (VOCs). The last analysis revealed the presence of eight chemical classes with aldehydes, esters, alcohols and acids as major compounds and allowed the separation of the trials FHS and OHS. Sensory attributes were significantly different for judges and pizzas and the most relevant differences were found for crust colour, presence of bubbles, resistance to tearing, crispness and chewiness. The overall assessment reached the highest scores for the mixed culture of OHS and FHS together.

*Keywords:* Heterofermentative metabolism; Lactic acid bacteria; Pizza dough; Sourdough; Volatile organic compounds

## 1. Introduction

Pizza represents the Italian cuisine in the world (Pagani et al., 2014) and is one of the emblems of glocal foods. The adjective “glocal” is referred to foods typical of a given area that exploited the process of globalization and are available worldwide (Bauman, 2005). The art of pizza spinning originated in ancient Naples (Ensminger et al., 1995). The first written description of a food similar to pizza (*dapes et adorea liba*) was reported by Virgil (first century b.C.) in the VII book of The Aeneid (Canali and Paratore, 2015). At the beginning of the XX century, pizza was produced and consumed only in Italy, but after the World War II pizza production was exported almost in all continents following the large-scale emigration of Italians (Caputo and Pugno, 2016). The production of Neapolitan pizza spinning is considered an art and, on last December 7$^{th}$ 2017, it received the recognition by UNESCO (United Nations Educational, Scientific and Cultural Organization) of an "intangible cultural heritage" and was added to the Representative List of the Intangible Cultural Heritage of Humanity (Ganguly, 2017) making pizza of worldwide relevance.

The use of sourdough in pizza production is an ancient practice (Chavan and Chavan, 2011). Since its first production, the fermentation process has been innovated only with regards to the leavening agent, mostly represented by baker's yeast in modern times. However, sourdough technology still remains particularly appreciated for the production of traditional pizza due to its greater advantages over baker's yeast, mainly represented by a higher as palatability, sensory quality and digestibility. The typical sourdough microorganisms are represented by *Lactobacillus* species, including all three metabolic groups known for LAB: obligate and facultative heterofermentative and obligate homofermentative species (Corsetti and Settanni, 2007).

*Lactobacillus* species, together with species of *Enterococcus*, *Leuconostoc* and *Weissella* genera, were reported to be the fermenting LAB of the sourdoughs used for Neapolitan pizza by Coppola et al. (1996). Among those isolates, some strains of *Lactobacillus plantarum* were found to induce the fastest acidification of experimental doughs (Coppola et al., 1998), evidencing a possible relevant role of the facultative heterofementative species (FHS) in pizza production. In general, obligately heterofermentative LAB ensure optimal bread production when sourdough is added as leavening agent (Alfonzo et al., 2016; Corona et al., 2016), due to the gas generation during heterolactic fermentation (Axelsson, 1998). Although, both pizza and bread are produced by sourdough technology, bread is mainly consumed unseasoned, while pizza undergoes topping before eating. As a matter of fact, the final characteristics might be influenced by the different microorganisms that operate during fermentation. With this in mind, the objective of the present work was to evaluate the impact of different FHS and obligate heterofermentative species (OHS) on the microbiological and the physicochemical properties of the sourdoughs and on the physical, chemical and sensory characteristics of the resulting pizzas.

## 2. Materials and methods

### *2.1. Starter cultures*

The strains used to produce pizzas belonged to the groups of obligate heterofermentative (*L. brevis* PON 200571, *L. rossiae* PON 100500 and *L. sanfranciscensis* PON 100336) and obligate heterofermentative (*L. curvatus* PON 100490, *L. graminis* PON 100244 and *L. plantarum* PON 100148) *Lactobacillus*. All these cultures, belonging to the culture collection of the Laboratory of Agricultural Microbiology (University of Palermo, Italy), were isolated from raw materials used in sourdough bread production (Alfonzo et al., 2013, 2017) or from mature sourdoughs (Ventimiglia et al., 2015).

All LAB were grown overnight at 30°C. *Lactobacillus sanfranciscensis* and *L. graminis* were reactivated in Sour Dough Bacteria (SDB) broth, while *Lactobacillus brevis*, *L. rossiae*, *L. plantarum* and *L. curvatus* in modified-MRS (mMRS) broth (Corsetti et al., 2008).

### *2.2. Sourdough production and propagation*

The strains were prepared individually after centrifugation of the overnight grown cultures at 5000 × *g* for 5 min, followed by two washing steps in Ringer's solution (Oxoid, Milan, Italy) and final re-suspension at the optical density 1.00 measured at 600 nm. Each single inoculum was propagated (1%, v/v) using the sterile flour extract (SFE) reported by Alfonzo et al. (2016). After incubation at 30°C for 24 h, the cultures were directly used for the mono-species trials (LB, LC, LG, LP, LR and LS, corresponding to *L. brevis*, *L. curvatus*, *L. graminis*, *L. plantarum*, *L. rossiae* and *L.*

*sanfranciscensis*, respectively) or mixed for the multi-strain inocula (OHS, the three obligate heterofermentative species; FHS, the three facultative heterofermentative species; OFHS, all six strains together). The sourdough F1, previously characterized by Ventimiglia et al. (2015), was used for the control (CTR) trial.

The initial doughs were prepared with a dough yield (weight of the dough/weight of flour × 100) of 160 by means of the mechanical mixer SilverCrest Bread Maker SBB 850 A1 (Kompernass GMBH, Bochum, Germany) for 15 min. Each dough (640 g) was obtained adding to 400 g of commercial tender wheat flour Type 0 (Conad, Bologna, Italia) 240 mL of the cell suspensions in mineral water to reach a final cell density of about $10^6$ CFU/g. Each dough (D) was then fermented for 21 h at 30°C into sterile cylindrical glass jars (Vetreria di Borgonovo, Borgonovo, Italy) to obtain the corresponding sourdough. All sourdoughs were refreshed as follows: 200 g of acidified doughs were mechanically mixed with 125 g of flour and 75 mL of water and fermented for 21 h at 30°C. The adaptation of the initial inocula was performed for six consecutive days corresponding to six refreshments (RI – RVI). RVI sourdough of each trial was then used for pizza dough (PD) fermentation performed following a classical regional recipe: sourdough (64 g) was mixed with flour (360 g), water (216 mL) and salt (7.2 g) and incubated at 30°C for 8 h. All trials were carried out in duplicate after two weeks.

*2.3. Analyses of sourdoughs*

The acidification process was obtained by pH measurement on 10 g of doughs/sourdoughs by direct immersion of the pH-meter (XS Instruments, Carpi, Italy) probe followed by titratation with 0.1 N NaOH. The determination of total titratable acidity (TTA), expressed as mL of NaOH/10 g, was performed after homogenization of the samples in distilled $H_2O$. The acidification was monitored analysing (in triplicate) all samples just after ingredient mixing ($T_0$), at 2 h-interval for the first 8 h, and then after 21 h.

The concentrations of lactic and acetic acid and their molar ratio (FQ, fermentation quotient) were determined at $T_0$ and $T_{21}$ for all pizza dough. Chemical determinations were carried out by high performances liquid chromatography (HPLC) as described by Alfonzo et al. (2013). Sourdough samples (10 g) were homogenised in distilled $H_2O$ (90 mL) and 10 mL of each suspension were used for analyses. Data were acquired and processed with the PerkinElmer software specific for HPLC instrument (TotalChrom Workstation 2008 rev. 6.3.2). Chemical determinations were carried out in triplicate.

Microbiological analyses of doughs and refreshment samples were carried out at $T_0$, $T_8$ and $T_{21}$, while pizza doughs were analysed at $T_0$ and $T_8$. The samples (15 g) were suspended 1:10 in Ringer's solution, homogenised by stomacher (BagMixer® 400; Interscience, Saint Nom, France) and serially diluted. Cell suspensions were analysed for total mesophilic count (TMC), LAB and total yeasts as described by Alfonzo et al. (2016). Plate counts were performed in duplicate.

The dominance of the added strains was verified only at the end of fermentation for the sixth sourdough refreshments and for pizza doughs. The colonies developed on SDB agar were collected, purified and characterized by randomly amplified polymorphic DNA (RAPD)-PCR technique as reported by Gaglio et al. (2017). The polymorphic profiles of the LAB isolated from each trial were compared to those of the pure cultures of the added strains.

*2.4. Analyses of pizzas*

The fermented pizza doughs were passed through a sheeting machine to reach the thickness of 1.5 mm indicated by Bernklau et al. (2017). Discs of ca. 20 g were obtained  with a circular stainless steel moulder (10 cm diameter). The unseasoned pizzas were baked in an oven equipped with a refractory stone (PizzaCLUB, ITALKERO SRL, Modena, Italy) at 310 (bottom heat) – 380°C (top heat) for 2 min.

After baking, pizzas were cooled at room temperature for 15 min and weighted. Colour was determined by the Chroma Meter CR-400C colorimeter (Minolta, Osaka, Japan) on five points of the upper and lower surface of two pizzas for each trial measuring the Hunter's scale L*, a* and b* parameters. Both pizza surfaces were also scanned at a resolution of 300 dpi in order to perform the analysis of the images saved in TIFF format. Each image was analysed with the ImageJ software (National Institutes Health, Bethesda, Md, USA), cropped to a square of 207 × 207 pixels (representing 15×15mm of each surface) and converted to grey-level image (8 bit). A binary image was obtained applying the Otsu's threshold algorithm in order to detect the particle information to get a detailed explanation of the overall morphology. With "area selection", for each pizza sample, the parameters area (white area and average area of white cells) and shape descriptors (circularity, roundness and solidity), reported by Ferreira and Rasband (2012), were acquired.

Volatile organic compounds (VOCs) emitted from pizzas were analysed by the solid phase micro-extraction (SPME) technique. Each sample (5 g) was transferred into a vial, added with 20 μL of 1-heptanol solution (35 mg/L 1-heptanol in 20% ethanol aqueous solution) and heated to 60°C before the headspace was collected and analysed following the methodology described by Alfonzo et al. (2013). The identification of the chemical compounds was performed as reported by Settanni et al. (2013). VOCs were expressed as relative peak areas (peak area of each compound/total area) × l00. All determinations were performed in triplicate.

*2.5. Sensory analysis*

The sensory attributes of pizzas (30 cm diameter), baked in a convection oven at 480 (top heat) – 440 °C (bottom heat) for 3 min, were evaluated as reported by Bernklau et al. (2017) without topping to analyse the crust and after topping with tomato sauce to test the central part.

The sensory analysis was performed with triangular portions of each pizza by a descriptive panel consisting of 14 tasters (seven women and seven men, ranging from 22 to 63 years old). The analysis was carried out according to the guidelines in the ISO 6658. The judges were instructed to test pizzas and were asked to score 20 descriptors including crust colour, presence and uniformity of bubbles, odour intensity, yeast odour and unpleasant odour of crust, resistance to tearing, crispness and chewiness of crust, sweet, salty, acid, bitter, taste persistency, aroma intensity and yeast aroma. The overall acceptability of the products was also evaluated. Quality was scored using a line scale anchored on the left (visual analogue scale) with dislike/low quality and on the right with like/high quality.

### *2.6. Statistical analyses*

The analysis of variance (ANOVA) test was applied to identify significant differences among data. The post-hoc Tukey's method was applied for pairwise comparison in case of microbial counts, organic acids, characteristics of pizza and sensory scores. Statistical significance was $P < 0.05$.

An explorative multivariate approach was also applied to investigate the relationships among data obtained from the different trials. The different trials were grouped by principal component analysis performed with data obtained from sourdoughs and pizzas. The number of principal factors was selected according to the Kaiser criterion and only factors with eigen-values higher than 1.00 were retained. All data were preliminary evaluated by the Barlett's sphericity test in order to check the statistically significant differences among samples within each data set.

All statistical analysis were achieved by using XLStat software version 2014.5.03. (Addinsoft, New York, USA) for excel.

## 3. Results and discussion

### *3.1. Monitoring of the acidification process*

The kinetics of pH for initial doughs, sourdoughs and pizza doughs (Fig. 1) showed a similar trend for all trials. The most consistent pH drop for doughs was recorder after 6 h of fermentation. The lowest pH values during the six refreshments were registered for the multiple combinations of *Lactobacillus* that included the obligate heterofermentative strains, in particular OFHS trial, while the highest pHs were recorded for control, LC and FHS trials. During pizza dough production, the trials OFHS, OHS, LS, LR and LP reached the lowest pHs after 8 h of fermentation.

The monitoring of the acidification process included also TTA determination (Fig. 2). The kinetics of TTA were correlated linearly with pHs for all trials; the increase of acidity of doughs, sourdoughs and pizza doughs corresponded to the decrease of pH. LC trial was confirmed to be the fermentation process characterized by the slowest acidification rate followed by LG trial. When the final pizza doughs were fermented, the highest TTA values at 8 h were reached by LR, OFHS, LS and CTR trials.

The concentration of the main organic acids produced by facultative and obligate heterofermentative LAB was determined only for pizza doughs at $T_0$ and $T_8$ (Table 1). Just after dough kneading, lactic acid was in the range 0.35 – 0.86 mg/g with the lowest value registered for LG PD and the highest for FHS PD. The levels of this acid increased consistently after 8 h until reaching 4.75 mg/g for the trial LS. The increase of lactic acid concentration of the doughs LC and LG were particularly low (1.55 and 2.05 mg/g, respectively). The last two trials, together with FHS trial, were also characterized by the lowest levels of acetic acid, while LR PD and OFHS PD displayed the highest concentration (0.85 mg/g) of this acid. In order to determine how lactic and acetic acid impacted the aroma profile of each dough, the FQ was calculated. This parameter was particularly high for all PDs started with facultative heterofermentative strains due to their low production of acetic acid. For all other trials, FQ value was almost in the range 1.5 – 4 that is considered to influence positively the final aromatic and textural properties of the doughs (Spicher, 1983). The values of pH, TTA and the concentration of lactic and acetic acids and the corresponding FQs of all trials including the obligate heterofermentative strains were comparable to the values registered for sourdoughs propagated at industrial level (Corona et al., 2016).

*3.2. Evolution of inoculums*

The microbial groups followed for initial doughs, sourdoughs and pizza doughs were LAB, TMC and yeasts (Fig. 3). The highest levels of all groups were registered for CTR triil at the beginning of the process (Fig. 3A). All selected *Lactobacillus* inocula were in the range of 6.52 – 7.60 Log

CFU/g. After 8 h of fermentation, the highest LAB levels were displayed by the trial FHS, but this trial was superseded in cell density by the doughs of trial OFHS at 21 h. During sourdough refreshments, LAB levels increase until almost 9 Log CFU/g for CTR and LB trials and exceeding 10 Log CFU/g in OFHS sourdoughs. The final pizza doughs confirmed the trend registered for all trials and the highest levels of LAB were detected for OHS and OFHS PDs (9.81 and 10.66 Log CFU/g, respectively). Similar levels of LAB were generally registered in mature sourdoughs produced at artisan and industrial level (Corona et al., 2016; Minervini et al., 2012; Ventimiglia et al., 2015). However, regarding pizza doughs, the levels of the LAB registered before baking in our study are higher than those reported in literature for artisanal sourdough pizza productions (Coppola et al., 1996).

The levels of TMC (Fig. 3B) were lower than those of LAB. Similar findings are reported by previous studies on the microbiological parameters of sourdoughs produced with selected LAB inoculums (Alfonzo et al., 2016; Corona et al., 2016), probably due to the lower nutrient availability of PCA in comparison to SDB, that is also characterized by a pH of 5.6 that favours the growth of sourdough LAB (Gänzle et al., 1998).

Contrarily to CTR control trial, the experimental trials were characterized by very low levels of yeasts (2.96 – 3.21 Log CFU/g) at the beginning of the process. These levels correspond to the levels of yeasts generally reported for wheat flour (Berghofer et al., 2003). The levels of this group increased during fermentation, but did not exceed $10^6$ CFU/g in control trial and reached 7.01 – 7.35 Log CFU/g after the 6$^{th}$ refreshment for all LAB started trials. This increase was previously observed by Corona et al. (2016) for experimental sourdoughs produced with different heterofermentative LAB strains. The ratio between yeasts and LAB detected in pizza doughs was almost 1:100 which is generally found for sourdoughs produced in Italy (Alfonzo et al., 2016; Valmorri et al., 2006).

The persistence of the added starters through the entire process of pizza dough production was evaluated by comparison of the RAPD patterns of the presumptive LAB isolated from SDB for the sourdoughs at the sixth refreshment and pizza doughs at the end of fermentation (Results not shown). The monitoring of the RAPD profiles of dominant strains was found to be useful to follow sourdough fermentation processes (Alfonzo et al., 2016; Corona et al., 2016). All mono-culture LAB trials were found to be dominated by the added strains. Regarding the multiple strain started sourdoughs, OHS trial showed similar levels of *L. sanfranciscensis* PON 100336 and *L. brevis* 200571, while *L. rossiae* PON 100500 was at 1 Log CFU/g lower, FHS trial showed a clear dominance of *L. plantarum* PON 100148, followed by *L. graminis* PON 100244, but *L. curvatus* PON 100490 was not detected among the highest dilutions. OFHS trial was dominated by *L. sanfranciscensis* PON 100336, *L. brevis* 200571 and *L. plantarum* PON 100148. The last three species are common in traditional type I sourdough processes (Lhomme et al., 2015; Minervini et al., 2012). RAPD profiles of the selected LAB used for experimental sourdoughs were not found associated to any LAB from CTR trial.

*3.3. Characteristics of pizzas*

The characteristics of pizzas are reported in Table 2. The weight loss of pizzas of the 10 trials were statistically different. However, the final weight of pizzas of the trial OFHS was similar to those of LC and LG pizzas and that of FHS to LP pizzas.  The lowest weight loss was shown by CTR trial (3.04 g).

All colour parameters were different among trials. The highest L* values were found for LR and OHS trials, while CTR pizzas were characterized by a consistently lower value in comparison to all other trials. Opposite results were found for a*, since CTR pizzas showed the highest value. Regarding b*, the values recorded for the obligate heterofermentative species alone or in combination were higher than those of the other trials, including CTR. These results showed that the colour parameters of pizzas are influenced by starter strains and their interaction as reported for sourdough breads (Corona et al., 2016; Settanni et al., 2013).

The image analysis allowed to evaluate the differences of pizza morphology among trials. In fact, white area % and the average area of white cells resulted statistically different. Regarding the last parameter, all three trials inoculated with the facultative heterofermentative *Lactobacillus*, that resulting from their combination and CTR were comparable. However, no statistically differences were found for the three shape descriptors. Circularity showed values higher than 0.80 for eight of the 10 trials, showing a high regularity of particles (Ferreira and Rasband, 2012).

Pizzas emitted eight classes of volatile compounds: acids, alcohols, aldehydes, esters, furans, ketones, phenols and terpenes. The dendrogram (Fig. 4) obtained from the cluster analysis and the heat map shows the differences in the concentrations of the 45 VOCs detected for the samples analysed. The chemical complexity of sourdough pizzas was similar to that of sourdough breads (Hansen and Schieberle, 2005; Salim-ur-Rehman et al., 2006). The concentrations of the VOCs among pizzas determined the grouping of the trials in two main clusters. LC, LG, LP and FHS formed the first cluster. In particular, the trials stared with the facultative heterofermentative strains were characterized by the highest levels of furfuraldehyde (22.08 – 25.12 %), benzaldehyde (8.31 – 11.09%), 2-ethylhexanol (2.68 – 3.56%) and phenylethanal (1.36 – 1.75 %). The second main cluster that included all other trials showed two sub-clusters, one with all obligate heterofermentative strains alone or in triple combination, characterized by the highest generation of benzyl acetate (12.25 – 18.57 %), acetic acid (16.20 – 18.25 %) and 3-methyl-1-butanol (2.86 – 4.03 %), and another one comprising CTR and OFHS, mainly characterized by the highest production of 3-phenylfuran, decanal, geranylacetone and 2,3-butanediol. Among the compounds found at the highest concentrations, acetic acid, a flavour enhancer (Molard et al., 1979), is due to the hetero-fermentation (Axelsson, 1998). The production of 3-methyl-1-butanol is responsible for the “fermented” flavour of sourdough breads (Salim-ur-Rehman et al., 2006) and is generally detected in presence of combination of heterofermentative LAB (Corona et al., 2016). Phenylethanal (floral-green aroma) is among the aldehyde compounds positively correlated with the aroma of wheat bread (Pico et al., 2015). Decanal is characterized by a typical aldehydic odour type, while benzaldehyde by fruity notes (Ripari et al. 2016). Geranylacetone is only occasionally found in sourdoughs (Ripari et al., 2016).

*3.4. Sensory attributes of pizzas*

All sensory attributes evaluated for pizzas were significantly different for judges and for the 10 trials (Table 3). The highest scores for crust colour were registered for CTR and OFHS trials. The presence of bubbles as well as their uniformity was scored at the highest levels in CTR pizzas. at highest in control flour pizzas, but the highest scores for were registered for FHS flour pizzas. No big differences were found for odour intensity among trials, even though the highest scores were reached by CTR pizzas. Yeast and unpleasant odour scores were low for all pizza samples. The highest values for resistance to tearing and crispness were registered for OFHS pizzas, while chewiness was registered at the highest scores for OHS trial. In general, no consistent differences were found for sweet and salty taste between crust and centre of pizzas, while acidity was more appreciated in the central part due to the presence of tomato. The highest scores for bitterness were reached by LS trial for crust and LR trial for centre. The taste persistency was scored at the highest levels in CTR pizzas for crust and OFHS pizzas for centre. Regarding the overall assessment, OFHS samples were those characterized by the highest appreciation.

*3.5. Multivariate analysis*

The results of PCA showed nine eigen-values were higher than 1 and the first four accounted for 83.58% of total variability. However, Factor 1 and 2 together explained 58.43% of total variability. For this reason, the 26 variables were expressed as linear combination of the first two factors.

The score plot (Fig. 5A) showed the far distance among the trials and confirmed the strict correlation between the facultative heterofermentative species and obligate heterofermentative species in single or multiple combinations which clustered into two main group. The highest differences was found for the CTR trial along Factor 1 which has the highest incidence on the total variability. Another difference along Factor 1 was showed by the trials performed with the highest complexity of the selected LAB (OFHS). As shown by the loading plot (Fig. 5B), the Factor 1 was mainly affected by acids, aldehydes, TTA, FQ, weight loss and phenol while the variability associated to Factor 2 was mainly explained by L*, WL, a*, texture and SDB.

## 4. Conclusions

The multivariate relationships based on microbiological, chemical, physical and sensory parameters indicated that the pizzas produced with different LAB inocula were different. In general, the higher quality parameters were registered in presence of obligate heterofermentative LAB, especially in combination, even though the highest scores at the overall assessment were reached by the OFHS pizzas produced with all selected LAB in mixed culture.

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**Table 1.** Production of organic acids by *Lactobacillus* species in single and multiple combination.

| Pizza doughs | Lactic acid (mg/g) | Acetic acid (mg/g) | Fermentation quotient |
|---|---|---|---|
| CTR $T_0$ | $0.51 \pm 0.07^{ef}$ | $0.14 \pm 0.03^{c}$ | 2.43 |
| CTR $T_8$ | $3.42 \pm 0.12^{c}$ | $0.57 \pm 0.06^{b}$ | 4.00 |
| LB $T_0$ | $0.36 \pm 0.03^{ef}$ | $0.12 \pm 0.01^{c}$ | 2.00 |
| LB $T_8$ | $4.15 \pm 0.03^{b}$ | $0.69 \pm 0.17^{ab}$ | 4.01 |
| LC $T_0$ | $0.45 \pm 0.10^{ef}$ | $0.03 \pm 0.01^{c}$ | 10.00 |
| LC $T_8$ | $1.55 \pm 0.16^{d}$ | $0.16 \pm 0.06^{c}$ | 6.46 |
| LG $T_0$ | $0.35 \pm 0.03^{f}$ | $0.04 \pm 0.01^{c}$ | 5.83 |
| LG $T_8$ | $2.05 \pm 0.27^{d}$ | $0.21 \pm 0.10^{c}$ | 6.51 |
| LP $T_0$ | $0.82 \pm 0.10^{ef}$ | $0.06 \pm 0.02^{c}$ | 9.11 |
| LP $T_8$ | $4.05 \pm 0.17^{b}$ | $0.26 \pm 0.10^{c}$ | 10.38 |
| LR $T_0$ | $0.65 \pm 0.10^{ef}$ | $0.21 \pm 0.06^{c}$ | 2.06 |
| LR $T_8$ | $3.98 \pm 0.07^{b}$ | $0.85 \pm 0.07^{a}$ | 3.12 |
| LS $T_0$ | $0.58 \pm 0.06^{ef}$ | $0.20 \pm 0.03^{c}$ | 1.93 |
| LS $T_8$ | $4.75 \pm 0.07^{a}$ | $0.75 \pm 0.06^{ab}$ | 4.22 |
| OHS $T_0$ | $0.67 \pm 0.17^{ef}$ | $0.19 \pm 0.10^{c}$ | 2.35 |
| OHS $T_8$ | $3.26 \pm 0.12^{c}$ | $0.78 \pm 0.06^{ab}$ | 2.79 |
| FHS $T_0$ | $0.86 \pm 0.15^{e}$ | $0.06 \pm 0.02^{c}$ | 9.56 |
| FHS $T_8$ | $4.14 \pm 0.20^{b}$ | $0.18 \pm 0.06^{c}$ | 15.33 |
| OFHS $T_0$ | $0.78 \pm 0.25^{ef}$ | $0.16 \pm 0.03^{c}$ | 3.25 |
| OFHS $T_8$ | $4.02 \pm 0.42^{b}$ | $0.85 \pm 0.16^{a}$ | 3.15 |
| Statistical significance | *** | *** | n.d. |

Abbreviations: CTR, control; LB, *L. brevis* PON 200571; LC, *L. curvatus* PON 100490; LG, *L. graminis* PON 100244; LP, *L. plantarum* PON 100148; LR, *L. rossiae* PON 100500; LS, *L. sanfranciscensis* PON 100336; OHS, obligate heterofermentative lactobacilli; FHS, facultative heterofermentative lactobacilli; OFHS obligate-facultative heterofermentative lactobacilli; n.d., not determined.

Results indicate mean values ± SD of six measurements (carried out in triplicate for two independent fermentations).

P value: ***, $P < 0.001$.

**Table 2.** Physical characteristics of unseasoned pizzas produced from sourdoughs fermented by *Lactobacillus* species in single and multiple combination

| Trial | Weight loss (g) | Color | | | White area % | Average area of white cells ($mm^2$) | Shape descriptors | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $L^*$ | $a^*$ | $b^*$ | | | Circularity | Roundness | Solidity |
| CTR | 3.04 ± 0.11$^{e}$ | 44.38 ± 0.97$^{e}$ | 1.94 ± 0.06$^{a}$ | 13.19 ± 0.41$^{e}$ | 36.46 ± 0.30$^{h}$ | 2.28 ± 0.11$^{e}$ | 0.87 ± 0.04$^{a}$ | 0.73 ± 0.04$^{a}$ | 0.92 ± 0.06$^{a}$ |
| LB | 4.06 ± 0.17$^{c}$ | 71.36 ± 2.06$^{abc}$ | 0.19 ± 0.03$^{c}$ | 18.39 ± 0.35$^{b}$ | 45.33 ± 0.41$^{c}$ | 3.35 ± 0.16$^{cd}$ | 0.74 ± 0.02$^{a}$ | 0.71 ± 0.01$^{a}$ | 0.84 ± 0.03$^{a}$ |
| LC | 5.35 ± 0.12$^{b}$ | 69.24 ± 1.78$^{abcd}$ | 0.25 ± 0.05$^{bc}$ | 15.63 ± 0.42$^{c}$ | 38.60 ± 0.33$^{g}$ | 2.25 ± 0.11$^{e}$ | 0.79 ± 0.02$^{a}$ | 0.72 ± 0.06$^{a}$ | 0.88 ± 0.02$^{a}$ |
| LG | 5.16 ± 0.14$^{b}$ | 65.10 ± 1.46$^{d}$ | 0.19 ± 0.02$^{c}$ | 14.52 ± 0.26$^{d}$ | 41.21 ± 0.39$^{e}$ | 2.28 ± 0.15$^{e}$ | 0.81 ± 0.03$^{a}$ | 0.80 ± 0.07$^{a}$ | 0.91 ± 0.04$^{a}$ |
| LP | 6.02 ± 0.13$^{a}$ | 68.29 ± 1.55$^{bcd}$ | 0.29 ± 0.04$^{bc}$ | 12.69 ± 0.21$^{e}$ | 49.31 ± 0.45$^{a}$ | 1.95 ± 0.05$^{e}$ | 0.85 ± 0.06$^{a}$ | 0.75 ± 0.05$^{a}$ | 0.85 ± 0.02$^{a}$ |
| LR | 3.65 ± 0.17$^{d}$ | 74.28 ± 2.53$^{a}$ | 0.22 ± 0.02$^{bc}$ | 17.56 ± 0.47$^{b}$ | 48.91 ± 0.43$^{a}$ | 3.95 ± 0.14$^{ab}$ | 0.85 ± 0.04$^{a}$ | 0.77 ± 0.08$^{a}$ | 0.92 ± 0.04$^{a}$ |
| LS | 3.80 ± 0.11$^{cd}$ | 72.65 ± 2.21$^{ab}$ | 0.26 ± 0.03$^{bc}$ | 17.64 ± 0.33$^{b}$ | 43.55 ± 0.41$^{d}$ | 4.09 ± 0.19$^{a}$ | 0.82 ± 0.03$^{a}$ | 0.77 ± 0.01$^{a}$ | 0.87 ± 0.03$^{a}$ |
| OHS | 3.88 ± 0.14$^{cd}$ | 73.92 ± 2.40$^{a}$ | 0.23 ± 0.01$^{bc}$ | 17.90 ± 0.42$^{b}$ | 47.49 ± 0.40$^{b}$ | 3.58 ± 0.16$^{bc}$ | 0.81 ± 0.16$^{a}$ | 0.74 ± 0.06$^{a}$ | 0.90 ± 0.08$^{a}$ |
| FHS | 5.96 ± 0.16$^{a}$ | 68.33 ± 1.68$^{bcd}$ | 0.24 ± 0.02$^{bc}$ | 13.42 ± 0.36$^{e}$ | 48.30 ± 0.43$^{ab}$ | 2.20 ± 0.19$^{e}$ | 0.86 ± 0.06$^{a}$ | 0.74 ± 0.07$^{a}$ | 0.90 ± 0.02$^{a}$ |
| OFHS | 5.25 ± 0.15$^{b}$ | 66.60 ± 1.67$^{cd}$ | 0.31 ± 0.05$^{b}$ | 20.16 ± 0.47$^{a}$ | 39.92 ± 0.45$^{f}$ | 2.96 ± 0.14$^{d}$ | 0.87 ± 0.02$^{a}$ | 0.77 ± 0.01$^{a}$ | 0.89 ± 0.01$^{a}$ |
| Statistical significance | *** | *** | *** | *** | *** | *** | N.S. | N.S. | N.S. |

Abbreviations: CTR, control; LB, *L. brevis* PON 200571; LC, *L. curvatus* PON 100490; LG, *L. graminis* PON 100244; LP, *L. plantarum* PON 100148; LR, *L. rossiae* PON 100500; LS, *L. sanfranciscensis* PON 100336; OHS, obligate heterofermentative lactobacilli; FHS, facultative heterofermentative lactobacilli; OFHS obligate-facultative heterofermentative lactobacilli.

Results indicate mean values± SD of six determinations (carried out in duplicate two independent productions).

Data within a column followed by the same letter are not significantly different according to Tukey's test

P value: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; N.S., not significant.

**Table 3.** Sensory evaluation of experimental seasoned pizzas produced from sourdoughs fermented by *Lactobacillus* species in single and multiple combination.

| Attributes | Trials | | | | | | | | | | SEM | Statistical significance | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CTR | LB | LC | LG | LP | LR | LS | OHS | FHS | OFHS | | Judges | Pizzas |
| Crust color | 2.36[a] | 2.31[a] | 1.95[cd] | 1.95[cd] | 2.28[ab] | 2.06[bc] | 2.22[ab] | 1.76[d] | 2.09[c] | 2.38[a] | 0.08 | ** | *** |
| Presence of bubbles | 5.85[a] | 4.96[ab] | 3.65[e] | 3.95[de] | 4.07[cde] | 5.15[ab] | 5.26[ab] | 4.89[bc] | 3.87[e] | 4.74[bcd] | 0.29 | ** | ** |
| Uniformity of bubbles | 3.33[a] | 2.84[bcd] | 3.30[a] | 3.21[ab] | 3.05[abc] | 2.66[de] | 2.46[e] | 2.74[cde] | 3.18[ab] | 3.17[ab] | 0.12 | * | ** |
| Odor intensity | 3.76[a] | 3.52[ab] | 2.36[f] | 2.54[ef] | 3.04[cde] | 3.35[abcd] | 3.26[abcd] | 3.25[bcd] | 2.88[de] | 3.43[abc] | 0.18 | ** | *** |
| Yeast odor | 1.63[bc] | 1.52[bcd] | 1.63[bc] | 1.33[de] | 1.26[e] | 1.80[ab] | 1.59[bc] | 1.39[cde] | 1.80[ab] | 1.95[a] | 0.09 | ** | ** |
| Unpleasant odor | 1.06[f] | 1.55[ab] | 1.10[ef] | 1.18[def] | 1.36[bcd] | 1.59[a] | 1.30[cde] | 1.51[abc] | 1.16[def] | 1.33[cd] | 0.08 | ** | ** |
| Resistance to tearing | 2.63[e] | 3.05[cde] | 3.62[ab] | 3.51[abc] | 3.78[ab] | 2.69[e] | 3.30[bcd] | 2.99[de] | 3.59[abc] | 3.91[a] | 0.18 | ** | ** |
| Crispness | 2.47[c] | 2.95[bc] | 3.65[ab] | 3.58[ab] | 4.20[a] | 3.02[bc] | 2.74[c] | 2.68[c] | 3.60[ab] | 4.11[a] | 0.25 | *** | ** |
| Chewiness | 3.94[ef] | 4.20[bcd] | 4.15b[cde] | 3.75[f] | 4.06[cde] | 4.31[bc] | 4.29[bc] | 4.61[a] | 3.99[def] | 4.36[ab] | 0.10 | * | ** |
| Taste of crust: | | | | | | | | | | | | | |
| Sweet | 3.28[b] | 3.08[bc] | 2.85[cd] | 2.74[d] | 2.76[d] | 2.95[cd] | 3.14[bc] | 2.94[cd] | 2.89[cd] | 3.62[a] | 0.11 | ** | ** |
| Salty | 2.44[a] | 2.36a[b] | 2.06[b] | 2.30a[b] | 2.18[ab] | 2.20[ab] | 2.34[ab] | 2.13[ab] | 2.26[ab] | 2.22[ab] | 0.13 | * | ** |
| Acid | 1.64[bc] | 1.82[ab] | 1.81[ab] | 1.72[bc] | 1.66[bc] | 1.86[ab] | 2.04[a] | 1.80[ab] | 1.61[bc] | 1.51[c] | 0.09 | * | * |
| Bitter | 1.56[cde] | 1.39[de] | 1.56[cde] | 1.29[f] | 1.69[bcd] | 1.95[ab] | 2.03[a] | 1.94[ab] | 1.57[cd] | 1.82[abc] | 0.10 | * | ** |
| Taste persistency | 6.28[a] | 5.15[cd] | 4.56[e] | 4.91[de] | 4.68[e] | 5.06[cde] | 5.59[bc] | 5.36[bcd] | 4.98[de] | 5.82[ab] | 0.19 | *** | *** |
| Aroma intensity | 4.14[ab] | 4.25[a] | 3.00[c] | 3.25[c] | 3.17[c] | 4.02[ab] | 3.84[b] | 3.89[ab] | 3.38[c] | 4.06[ab] | 0.16 | ** | ** |
| Yeast aroma | 1.71[ab] | 1.36[cd] | 1.85[a] | 1.39[cd] | 1.45[cd] | 1.52[bc] | 1.23[d] | 1.48[bcd] | 1.62[abc] | 1.57[bc] | 0.11 | * | * |
| Taste of center: | | | | | | | | | | | | | |
| Sweet | 3.23[a] | 3.20[a] | 2.95[ab] | 3.16[a] | 3.04[ab] | 3.01[ab] | 3.12[ab] | 2.61[b] | 3.37[a] | 3.40[a] | 0.19 | ** | * |
| Salty | 2.83[ab] | 2.65[abcd] | 2.51[bcd] | 2.39[d] | 2.45[cd] | 2.48[bcd] | 2.59[abcd] | 2.41[d] | 2.89[a] | 2.78[abc] | 0.13 | * | * |
| Acid | 3.02[a] | 2.70[ab] | 2.85[ab] | 2.75[ab] | 2.92[ab] | 2.58[b] | 2.78[ab] | 2.89[ab] | 2.96[ab] | 2.74[ab] | 0.15 | * | * |
| Bitter | 1.81[b] | 1.96[ab] | 2.05[ab] | 2.09[ab] | 2.18[a] | 2.23[a] | 2.08[ab] | 2.06[ab] | 2.12[ab] | 1.94[ab] | 0.12 | * | * |
| Taste persistency | 4.04[b] | 4.22[ab] | 4.15[ab] | 4.36[ab] | 4.29[ab] | 4.02[b] | 4.00[b] | 4.09[b] | 4.25[ab] | 4.63[a] | 0.18 | * | * |
| Overall assessment | 4.59[abc] | 3.85[def] | 3.96[def] | 3.68[ef] | 4.38[bcd] | 4.12[cde] | 4.68[ab] | 3.55[f] | 4.68[ab] | 4.97[a] | 0.20 | *** | ** |

Abbreviations: CTR, control; LB, *L. brevis* PON 200571; LC, *L. curvatus* PON 100490; LG, *L. graminis* PON 100244; LP, *L. plantarum* PON 100148; LR, *L. rossiae* PON 100500; LS, *L. sanfranciscensis* PON 100336; OHS, obligate heterofermentative lactobacilli; FHS, facultative heterofermentative lactobacilli; OFHS obligate-facultative heterofermentative lactobacilli.
P value: *, P < 0.05; **, P < 0.01; ***, P < 0.001.
Result indicate mean value.
Data within a line followed by the same letter are not significantly different according to Tukey's test.

**Legend to figures**

**Fig. 1.** Evolution of pH during dough preparation, sourdough propagation and pizza dough production. Abbreviations: D, dough; R, refreshment; PD, pizza dough; CTR, control; LB, *L. brevis* PON 200571; LC, *L. curvatus* PON 100490; LG, *L. graminis* PON 100244; LP, *L. plantarum* PON 100148; LR, *L. rossiae* PON 100500; LS, *L. sanfranciscensis* PON 100336; OHS, obligate heterofermentative lactobacilli; FHS, facultative heterofermentative lactobacilli; OFHS obligate-facultative heterofermentative lactobacilli.

**Fig. 2.** Evolution of TTA during dough preparation, sourdough propagation and pizza dough production. Abbreviations: D, dough; R, refreshment; PD, pizza dough; CTR, control; LB, *L. brevis* PON 200571; LC, *L. curvatus* PON 100490; LG, *L. graminis* PON 100244; LP, *L. plantarum* PON 100148; LR, *L. rossiae* PON 100500; LS, *L. sanfranciscensis* PON 100336; OHS, obligate heterofermentative lactobacilli; FHS, facultative heterofermentative lactobacilli; OFHS obligate-facultative heterofermentative lactobacilli.

**Fig. 3.** Levels of microorganisms during dough preparation, sourdough propagation and pizza dough production. A, lactic acid bacteria; B, total mesophilic counts; C, yeasts. Abbreviations: D, dough; R, refreshment; PD, pizza dough; CTR, control; LB, *L. brevis* PON 200571; LC, *L. curvatus* PON 100490; LG, *L. graminis* PON 100244; LP, *L. plantarum* PON 100148; LR, *L. rossiae* PON 100500; LS, *L. sanfranciscensis* PON 100336; OHS, obligate heterofermentative lactobacilli; FHS, facultative heterofermentative lactobacilli; OFHS obligate-facultative heterofermentative lactobacilli.

**Fig. 4.** Distribution of the volatile organic compounds emitted from pizzas obtained from sourdoughs fermented by *Lactobacillus* species in single and multiple combination. The double hierarchical dendrogram is based on the values of VOCs. The heat map plot depicts the relative percentage of each compound within each pizza. Abbreviations: D, dough; R, refreshment; PD, pizza dough; CTR, control; LB, *L. brevis* PON 200571; LC, *L. curvatus* PON 100490; LG, *L. graminis* PON 100244; LP, *L. plantarum* PON 100148; LR, *L. rossiae* PON 100500; LS, *L.*

*sanfranciscensis* PON 100336; OHS, obligate heterofermentative lactobacilli; FHS, facultative heterofermentative lactobacilli; OFHS obligate-facultative heterofermentative lactobacilli.

**Fig. 5.** Score plot (A) and loading plot (B) resulting from principal component analysis on 26 variable groups determined on fermented pizza doughs, baked unseasoned pizzas and final seasoned products. Abbreviations: FQ, fermentation quotient; PCA, plate count agar; WL, Wallerstein laboratory medium; SDB, sourdough bacteria medium; TTA, total titratable acidity; CTR, control; LB, *L. brevis* PON 200571; LC, *L. curvatus* PON 100490; LG, *L. graminis* PON 100244; LP, *L. plantarum* PON 100148; LR, *L. rossiae* PON 100500; LS, *L. sanfranciscensis* PON 100336; OHS, obligate heterofermentative lactobacilli; FHS, facultative heterofermentative lactobacilli; OFHS obligate-facultative heterofermentative lactobacilli.

**Fig. 1.**

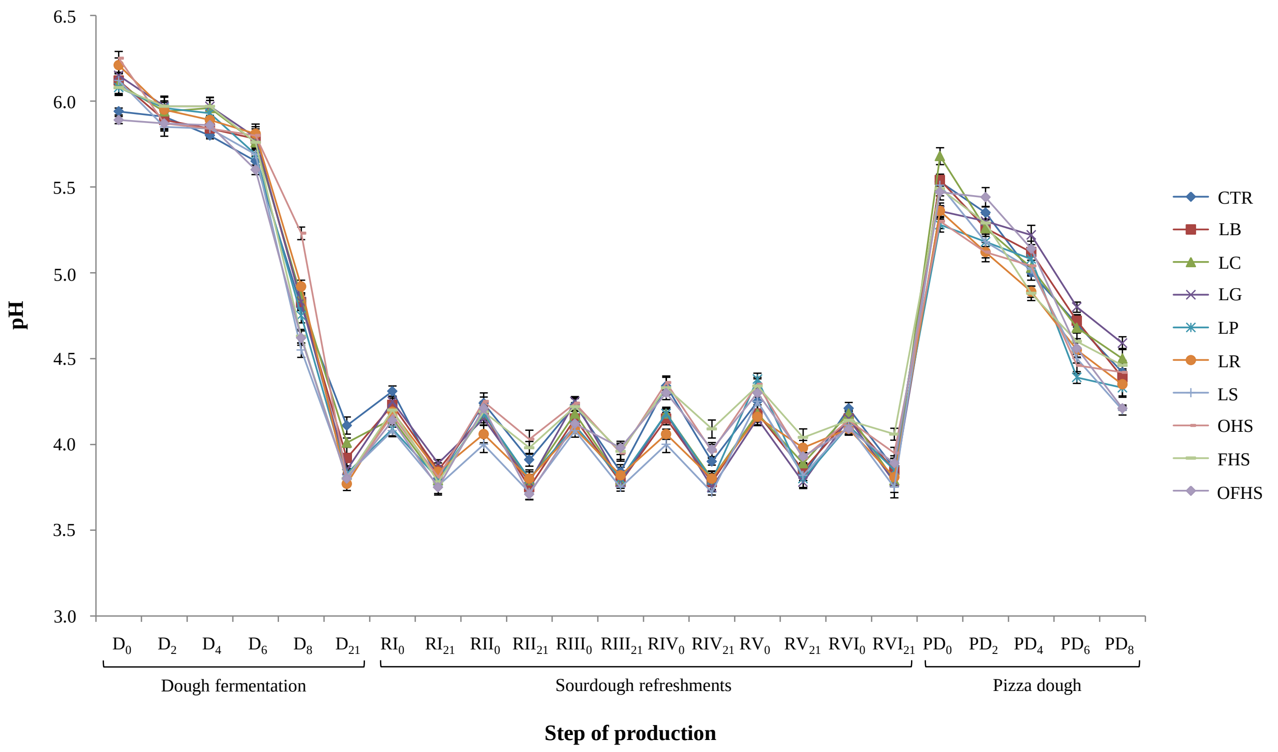
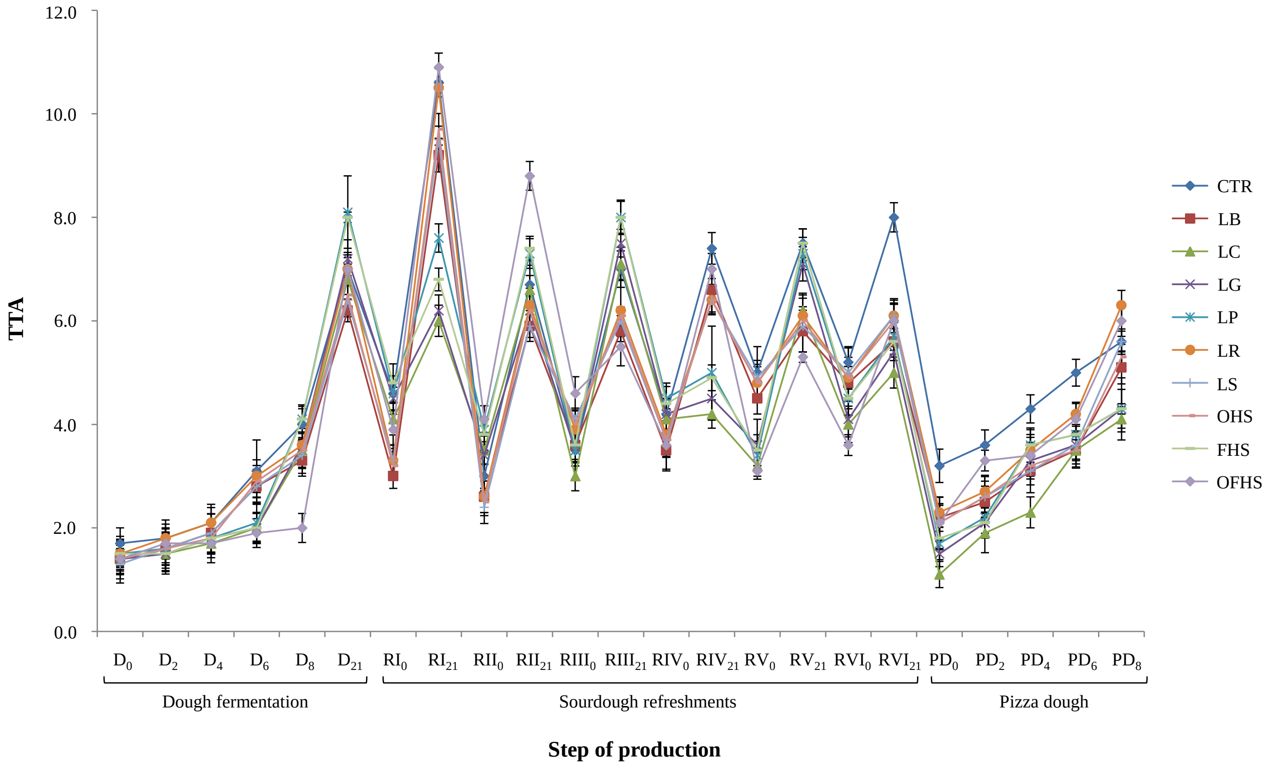

6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
pH
$D_0$ $D_2$ $D_4$ $D_6$ $D_8$ $D_{21}$ $RI_0$ $RI_{21}$ $RII_0$ $RII_{21}$ $RIII_0$ $RIII_{21}$ $RIV_0$ $RIV_{21}$ $RV_0$ $RV_{21}$ $RVI_0$ $RVI_{21}$ $PD_0$ $PD_2$ $PD_4$ $PD_6$ $PD_8$
Dough fermentation
Sourdough refreshments
Pizza dough
Step of production
CTR
LB
LC
LG
LP
LR
LS
OHS
FHS
OFHS

**Fig. 2.**



TTA
12.0
10.0
8.0
6.0
4.0
2.0
0.0
$D_0$ $D_2$ $D_4$ $D_6$ $D_8$ $D_{21}$ $RI_0$ $RI_{21}$ $RII_0$ $RII_{21}$ $RIII_0$ $RIII_{21}$ $RIV_0$ $RIV_{21}$ $RV_0$ $RV_{21}$ $RVI_0$ $RVI_{21}$ $PD_0$ $PD_2$ $PD_4$ $PD_6$ $PD_8$
Dough fermentation
Sourdough refreshments
Pizza dough
Step of production
CTR
LB
LC
LG
LP
LR
LS
OHS
FHS
OFHS

**Fig. 3.**

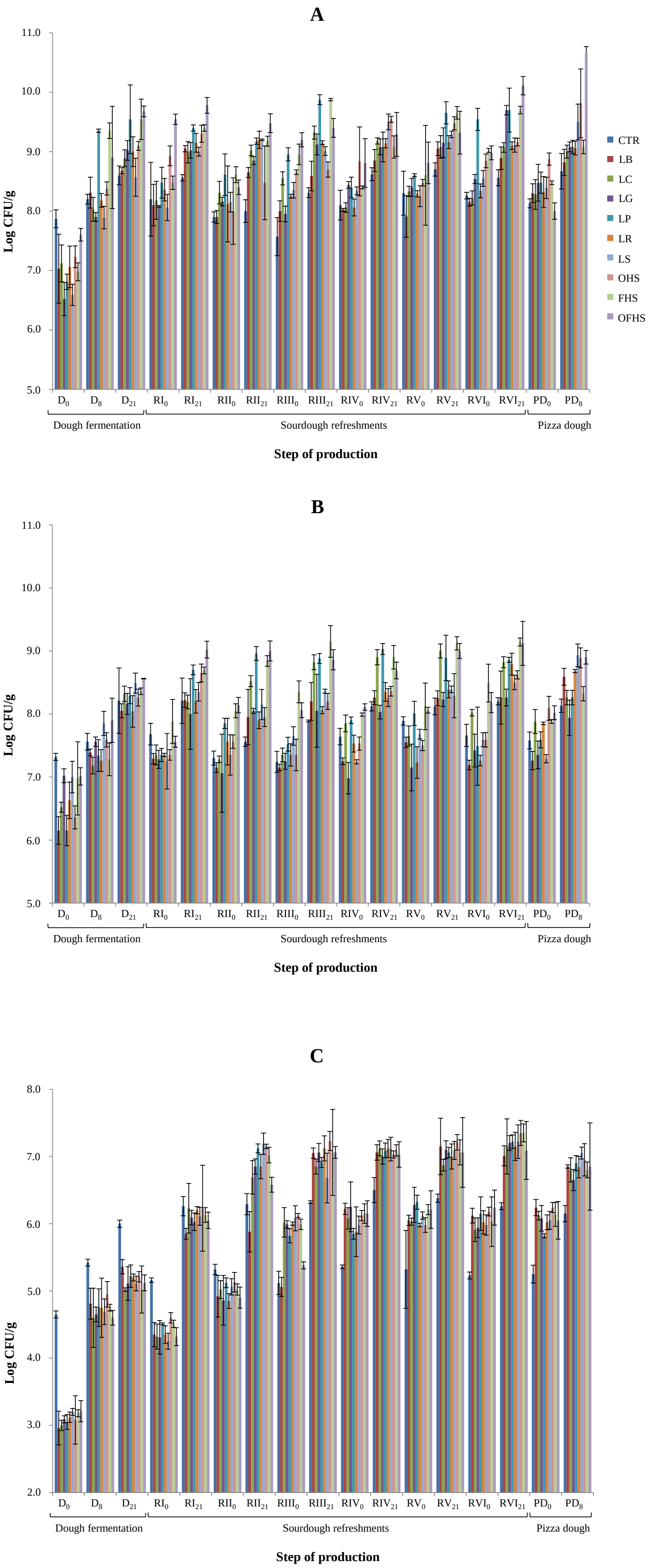

A
B
C
Log CFU/g
CTR
LB
LC
LG
LP
LR
LS
OHS
FHS
OFHS
$D_0$ $D_8$ $D_{21}$ $RI_0$ $RI_{21}$ $RII_0$ $RII_{21}$ $RIII_0$ $RIII_{21}$ $RIV_0$ $RIV_{21}$ $RV_0$ $RV_{21}$ $RVI_0$ $RVI_{21}$ $PD_0$ $PD_8$
Dough fermentation
Sourdough refreshments
Pizza dough
Step of production

**Fig. 4.**

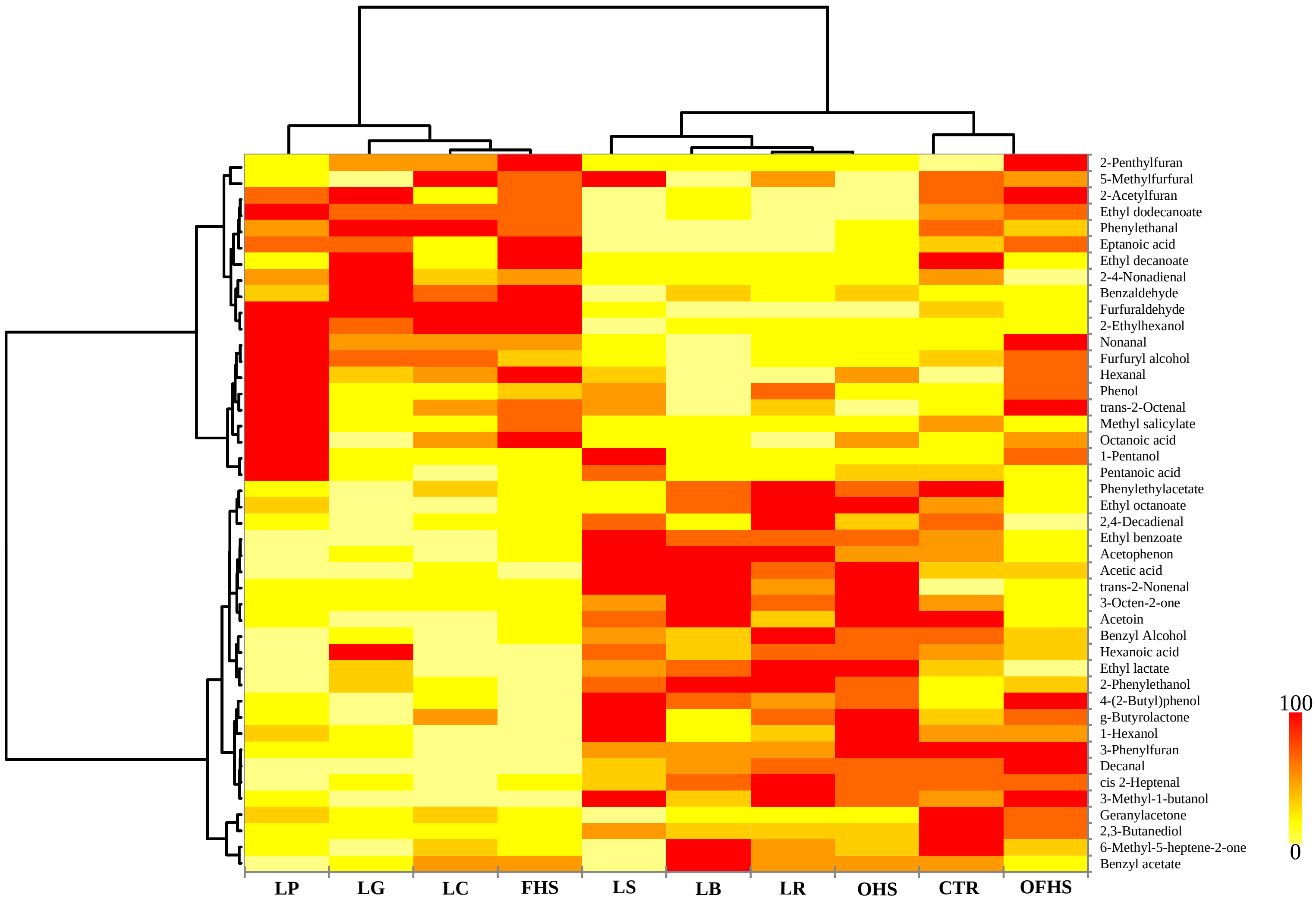
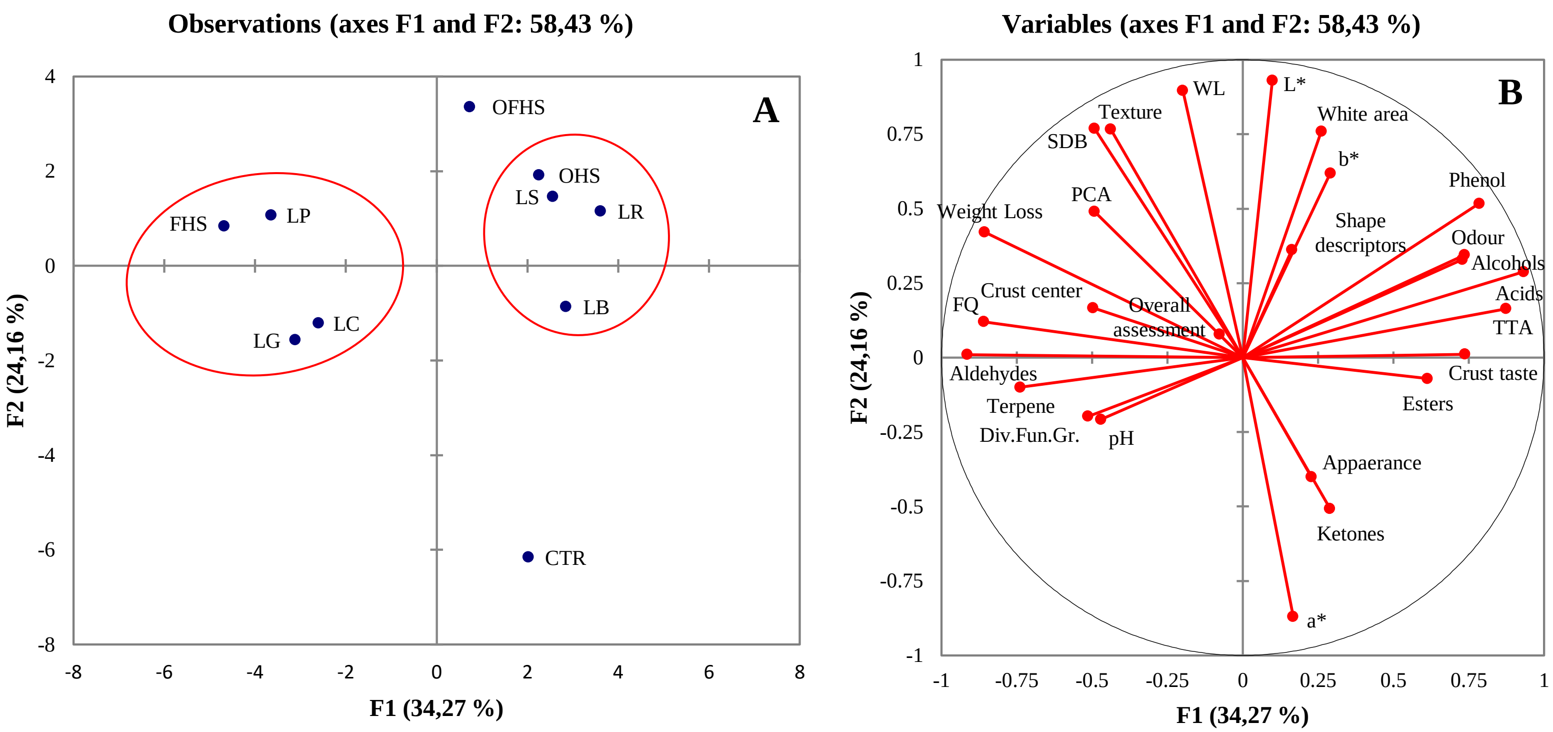

2-Penthylfuran
5-Methylfurfural
2-Acetylfuran
Ethyl dodecanoate
Phenylethanal
Eptanoic acid
Ethyl decanoate
2-4-Nonadienal
Benzaldehyde
Furfuraldehyde
2-Ethylhexanol
Nonanal
Furfuryl alcohol
Hexanal
Phenol
trans-2-Octenal
Methyl salicylate
Octanoic acid
1-Pentanol
Pentanoic acid
Phenylethylacetate
Ethyl octanoate
2,4-Decadienal
Ethyl benzoate
Acetophenon
Acetic acid
trans-2-Nonenal
3-Octen-2-one
Acetoin
Benzyl Alcohol
Hexanoic acid
Ethyl lactate
2-Phenylethanol
4-(2-Butyl)phenol
g-Butyrolactone
1-Hexanol
3-Phenylfuran
Decanal
cis 2-Heptenal
3-Methyl-1-butanol
Geranylacetone
2,3-Butanediol
6-Methyl-5-heptene-2-one
Benzyl acetate
LP
LG
LC
FHS
LS
LB
LR
OHS
CTR
OFHS
100
0

**Fig. 5.**


Observations (axes F1 and F2: 58,43 %)
A
OFHS
OHS
LS
LR
LB
FHS
LP
LC
LG
CTR
F1 (34,27 %)
F2 (24,16 %)
Variables (axes F1 and F2: 58,43 %)
B
WL
L*
Texture
SDB
White area
b*
PCA
Phenol
Weight Loss
Shape
descriptors
Odour
Alcohols
Acids
Crust center
FQ
Overall
assessment
TTA
Aldehydes
Crust taste
Esters
Terpene
Div.Fun.Gr.
pH
Appearance
Ketones
a*
F1 (34,27 %)
F2 (24,16 %)